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INFECTIOUS DISEASE

Transplacental Transmission of Ovine Herpesvirus 2 in Cattle with Sheep-associated Malignant Catarrhal Fever

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Summary

Sheep-associated malignant catarrhal fever (SA-MCF) is an important infectious disease of ruminants worldwide that is caused by ovine herpesvirus 2 (OvHV-2). OvHV-2 is transmitted predominantly by contact between infected and susceptible hosts, while the documentation of vertical transmission is rare. This report presents the pathological and molecular findings associated with transplacental transmission of OvHV-2 in cattle. Two Girolanda cows with corneal oedema, lethargy, mucopurulent nasal discharge and ulcerative stomatitis died spontaneously; one of these was pregnant with a 4-month-old fetus. Significant pathological findings included widespread lymphoplasmacytic necrotizing vasculitis and lymphoplasmacytic accumulations in several organs of both cows and the fetus. A polymerase chain reaction that targeted the tegument protein gene of OvHV-2 amplified viral DNA from the brain of the pregnant cow and her fetus, as well as from the kidney of the pregnant cow. The pathological findings observed in the cow and her fetus, together with the presence of OvHV-2 DNA in tissues of these animals, are suggestive of transplacental transmission of OvHV-2 in SA-MCF in cattle.

Keywords: cattle; malignant catarrhal fever; ovine herpesvirus 2; transplacental transmission

Introduction

Malignant catarrhal fever (MCF) is a fatal disease that affects domestic cattle, wild ruminants and occasionally pigs. MCF is caused by members of the genus *Macavirus* (Davison *et al.*, 2009), subfamily *Gammaherpesvirinae* (Russell *et al.*, 2009; O'Toole and Li, 2014). MCF has two principal epidemiological and clinical manifestations. The first is induced by alcelaphineherpesvirus 1 (AlHV-1), which uses wildebeest (*Connochaetes gnu* and *Connochaetes taurinus*) as a carrier and occurs predominantly within the African continent. The second is caused by ovine herpesvirus 2 (OvHV-2), which occurs outside the African continent and affects cattle, bison and deer, although sheep are the recognized carriers (Brown *et al.*, 2007; Russell *et al.*, 2009). Consequently, these manifestations are referred to as wildebeest associated (WA-MCF) and sheep-associated (SA-MCF) MCF, respectively (Brown *et al.*, 2007). However, other forms of MCF are described in several ruminant hosts (O'Toole and Li, 2014).

SA-MCF occurs worldwide (Russell *et al.*, 2009), affects a wide range of mammalian hosts (O'Toole and Li, 2014), and is endemic in Brazil, occurring in all geographical regions of the country, including the south (Rech *et al.*, 2005; Headley *et al.*, 2013a), midwest (Costa *et al.*, 2009) and northeast (Macêdo

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et al., 2007; Headley *et al.*, 2012). Transmission of OvHV-2 to susceptible animals is predominantly horizontal (Russell *et al.*, 2009), with nasal dissemination being demonstrated experimentally (Li *et al.*, 1998; Nishimori *et al.*, 2004). Vertical transmission has been suspected due to the detection of antibodies in virus-free and gnotobiotic lambs (Rossiter, 1981) and the identification of AlHV-1 from the fetus of wildebeest (Plowright, 1965). Although reports confirming the vertical transmission of OvHV-2 in cattle are sparse, viral DNA has been detected in an asymptomatic calf born to a cow with SA-MCF (O'Toole *et al.*, 1997). The present report describes the pathological and molecular findings associated with transplacental transmission of OvHV-2 in cattle.

Materials and Methods

Clinical History and Necropsy Examination

In late October 2013, a 3-year-old Girolanda cow (case 1) from the outskirts of the city of Cuiabá, Mato Grosso, central west Brazil, with clinical manifestations of profuse salivation, apparent blindness and anorexia was admitted to the Veterinary Teaching Hospital (VTH), Universidade de Cuiabá. Clinical evaluation revealed corneal opacity, lacrimation and severe, bilateral, mucopurulent nasal discharge (Fig. 1), fever, ruminal hypermotility, lethargy and ulcerative lesions in the oral cavity. In addition, the cow was carrying a 4-month-old fetus. The clinical condition of the cow deteriorated rapidly and the animal died spontaneously.

A 5-year-old cow (case 2) from the same farm was admitted to the VTH in early May 2013 with a history of bilateral ocular impairment that had reportedly started as keratoconjunctivitis 40 days earlier. This cow also died spontaneously. The interval between the onset of clinical manifestations and death of these cows was 7–40 days. An on-site visit to the farm revealed that there were 250 cows, a small flock of sheep reared for domestic consumption and intermingling between these species. The owner reported that all cows were maintained on pastures containing *Brachiaria brizantha* and were supplemented with corn silage and a soya bean-based ration, while water was supplied *ad libitum* at drinking troughs.

Both animals were submitted for routine necropsy examination soon after death. Tissue samples from the brain, lungs, kidneys, liver and myocardium of both cows and from the myocardium, lungs, kidneys and liver of the fetus were fixed in 10% neutral buffered formalin and processed routinely. Fresh tissue samples from the pregnant cow (brain and kidney) and fetus (brain) were kept at −80°C until used for molecular diagnostics.

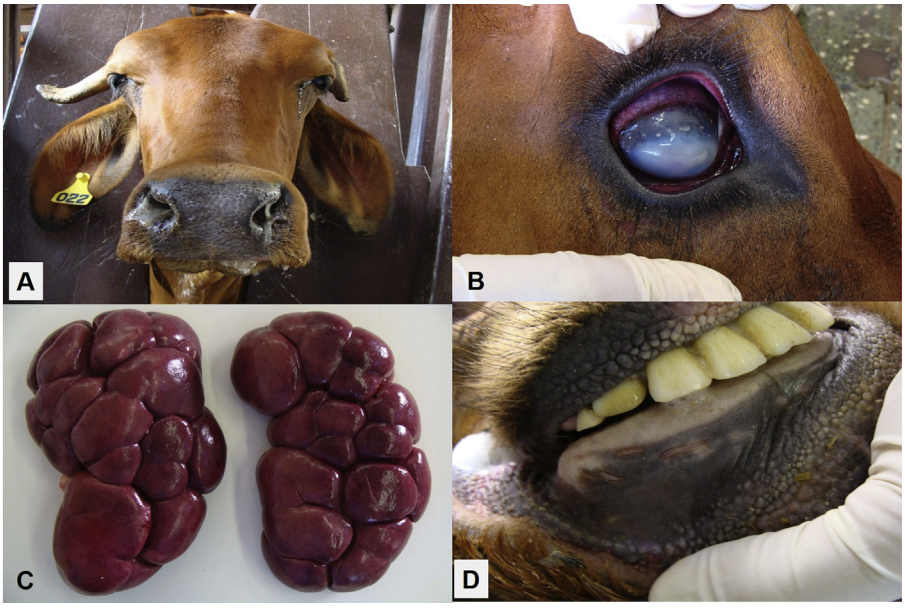

Fig. 1. Case 1 had mucopurulent nasal secretion (A) corneal oedema of the right ocular globe (B), fibrinopurulent bronchopneumonia, petechial haemorrhages within the mesenteric, thoracic and pleural surfaces, ulcerative stomatitis and rhinitis, pulmonary oedema, lymphadenomegaly and bilateral multifocal haemorrhagic nephritis (C). The fetus carried by this cow had normal external appearance and measured 30 cm in crown–rump length. Case 2 had bilateral corneal oedema, ulcerative stomatitis (D) and abomasitis.

Molecular Investigation

DNA extracted from the fresh tissue samples (Boom *et al.*, 1990) was used in polymerase chain reaction (PCR) assays designed to amplify specific regions of the OvHV-2 tegument protein gene (Baxter *et al.*, 1993), the glycoprotein C gene of bovine herpesvirus (BoHV)-1 and BoHV-5 (Claus *et al.*, 2005) and the 16S rRNA gene of *Histophilus somni* (Angen *et al.*, 1998). Positive controls consisted of DNA from other cases known to be infected with OvHV-2 (Headley *et al.*, 2012), prototype strains of BoHV-1 (Los Angeles) and BoHV-5 (AA-Par) amplified in Madin–Darby bovine kidney cells (Claus *et al.*, 2005) and *H. somni* (Headley *et al.*, 2013b). Nuclease-free water (Invitrogen Corporation, Carlsbad, California, USA) was used as a negative control in the PCR assays. All PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and examined under ultraviolet light.

The amplified PCR products were then purified (illustra GFX PCR DNA and Gel Band Purification Kit; GE Healthcare, Little Chalfont, Buckinghamshire, UK) and submitted for direct sequencing using the forward and reverse primers. The partial nucleotide sequences obtained were compared initially by the BLAST programme (http://www.ncbi.nlm.nih.gov/BLAST) with similar sequences deposited in GenBank. Sequence alignment and a phylogenetic tree were created using MEGA 6 (Tamura *et al.*, 2011), after which model selection indicated the Jukes–Cantor model as being the most appropriate for determination of the phylogenetic relationship with the maximum likelihood method. An initial tree for the heuristic search was obtained by applying the neighbour-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood approach. An identity matrix of the nucleotide sequences was generated by using the software BioEdit 7.2 (Hall, 1999).

Results

Pathological Findings

Case 1 had corneal oedema of the right ocular globe (Fig. 2), fibrinopurulent bronchopneumonia, petechial haemorrhages within the mesenteric, thoracic and pleural surfaces, ulcerative stomatitis and rhinitis, pulmonary oedema, lymphadenomegaly and bilateral multifocal haemorrhagic nephritis (Fig. 3). The fetus carried by this cow had normal external appearance and measured 30 cm in crown–rump length. Case 2 had bilateral corneal oedema, ulcerative stomatitis and abomasitis.

Histopathological evaluation of the tissues from both cows revealed lymphoplasmacytic, necrotizing vasculitis within the kidneys, carotid rete mirabile, brain, lymph nodes and lungs (Figs. 2A–C). Case 1 also had lymphoplasmacytic meningoencephalitis and nephritis, and purulent bronchopneumonia. Significant histopathological findings within the fetus

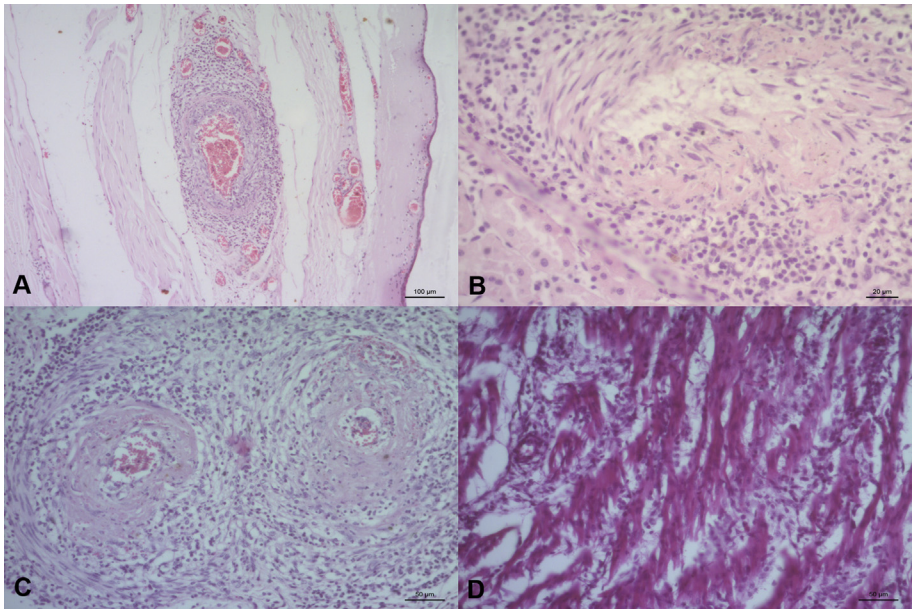

Fig. 2. Histopathological features of sheep associated-malignant catarrhal fever in a cow and fetus. Necrotizing lymphoplasmacytic vasculitis in the eye (A), kidney (B) and carotid rete mirabile (C) of the cow. (D) Fetal lymphoplasmacytic myocarditis. Haematoxylin and eosin. Bars: A, 100 μm; B, 20 μm; C–D, 50 μm.

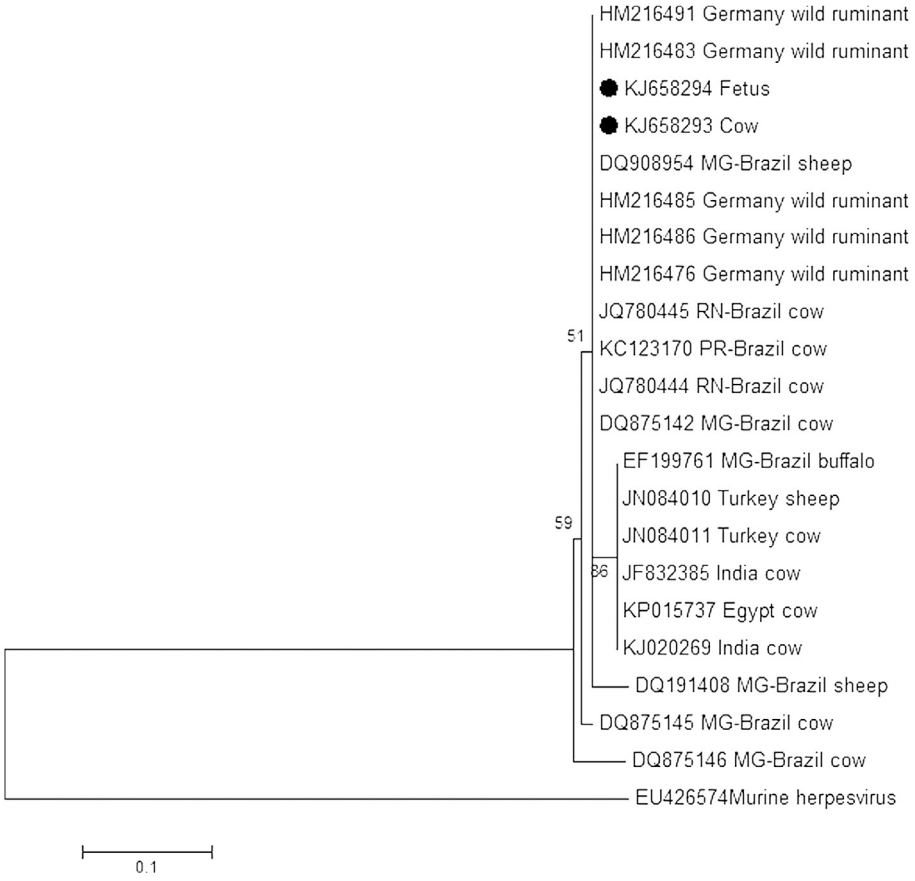

Fig. 3. This phylogenetic tree was created based on amplified sequences of the tegument protein gene of selected strains of ovine herpesvirus 2. The evolutionary history was inferred by using the maximum likelihood method based on the Jukes–Cantor model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site; the analysis involved 22 nucleotide sequences. The GenBank accession numbers and the geographical origin of the sequences used are given. The sequences derived from this study are highlighted (black dots). Murine herpesvirus was used as the out-group. MG, Minas Gerais; RN, Rio Grande do Norte; PR, Paraná; SP, São Paulo.

included lymphoplasmacytic necrotizing vasculitis and myocarditis (Fig. 2D), and accumulations of lymphoplasmacytic inflammatory cells within the kidneys and portal regions of the liver.

Molecular Characterization of OvHV-2

The PCR assay amplified the desired amplicons of the tegument protein gene of OvHV-2 from tissues of case 1 (brain and kidney), case 2 (kidney) and from the fetus (brain). Direct sequencing was applied to PCR products derived from case 1 (kidney) and the fetus (brain). Initial BLAST analyses revealed that these sequences (GenBank accession numbers KJ658293, cow; KJ658294, fetus) shared 98–99% identity with similar sequences deposited in GenBank. The phylogenetic tree clustered the two strains of OvHV-2 with other OvHV-2 viral sequences identified in ruminants from Brazil and wild ruminants from Germany (Fig. 3). Moreover, the generated sequence identity matrix and sequence alignment demonstrated that the nucleotide sequences of these two strains (case 1 and her fetus) were 100% identical. The bovine herpesvirus 1 (BoHV-1), BoHV-5 and *H. somni* PCR assays yielded negative results.

Discussion

The clinical and pathological findings in these two cows are similar to those frequently observed in cattle with SA-MCF (Brown *et al*., 2007; O'Toole and Li, 2014) and represent what is known as the 'head and eye' form of MCF (Russell *et al*., 2009; Li *et al*., 2014). The necrotizing lymphoplasmacytic vasculitis observed in multiple tissues of the two cows and the fetus, associated with the infiltration of lymphoplasmacytic inflammatory cells into several tissues of these animals, are the hallmarks of SA-MCF, and hence are characteristic diagnostic features of this disease (Brown *et al*., 2007; Russell *et al*.,

2009; Li *et al.*, 2014; O'Toole and Li, 2014). Amplification and sequencing of specific fragments of the tegument protein gene of OvHV-2 from the brain of case 1 and its fetus confirmed the likely participation of this pathogen in the development of the lesions observed in these animals (Li *et al.*, 2014). Similar findings of SA-MCF have been described in cattle (Headley *et al.*, 2012, 2013a) and buffalos (Costa *et al.*, 2009) from endemic regions of Brazil. The negative PCR results excluded the participation of BoHV-1 and BoHV-5 in the pathogenesis of these lesions. *H. somni*, which was recently described as an abortifacient (Headley *et al.*, 2015b) and neurological (Headley *et al.*, 2015a) agent, and associated with respiratory disease (Headley *et al.*, 2014) in cattle from Brazil, was discarded as a possible aetiological agent due to the negative PCR findings. Taken together, these findings suggest the transplacental dissemination of OvHV-2 from the clinically affected pregnant cow to the fetus.

Transplacental dissemination of OvHV-2 has been observed in an asymptomatic calf that contained viral DNA, but showed no clinical manifestations of MCF (O'Toole *et al.*, 1997). In addition, vertical transmission of OvHV-2 was inferred in virus-free and gnotobiotic lambs (Rossiter, 1981) and AlHV-1 was reported in the fetus of wildebeest (Plowright, 1965). OvHV-2 DNA or antibody was not detected in four fetuses born from MCF-positive ewes that were infected experimentally (Li *et al.*, 1998). Contact between infected and susceptible animals is the most important means of dissemination of MCF (Russell *et al.*, 2009; O'Toole and Li, 2014), while transplacental transmission appears to be a rare feature of SA-MCF in cattle (Li *et al.*, 1998). Consequently, the clinical significance of these findings relative to the dissemination of SA-MCF in cattle is unknown, but the possibility of transplacental infection cannot be overlooked.

Epidemiological data suggest that these cows were probably infected due to intermingling with asymptomatic sheep maintained on the farm. In Brazil, outbreaks of MCF are predominantly associated with contact with sheep (Rech *et al.*, 2005; Macêdo *et al.*, 2007; Costa *et al.*, 2009; Headley *et al.*, 2012), but cases without the concomitant presence of sheep (Macêdo *et al.*, 2007; Headley *et al.*, 2013a) have also been described. These epidemiological features are in accord with the worldwide distribution of SA-MCF (Li *et al.*, 2014). SA-MCF is of moderate economic importance to the Brazilian cattle industry, with a reported morbidity of 1–4% (Costa *et al.*, 2009; Headley *et al.*, 2013a) and estimated mortality of 83–100% (Rech *et al.*, 2005; Macêdo *et al.*, 2007). In this outbreak, the morbidity was 5% (2/250) with 100% mortality. The reduced morbidity and elevated mortality observed in SA-MCF of cattle from Brazil is similar to that described in other countries (Russell *et al.*, 2009; O'Toole and Li, 2014). Additionally, the 'head and eye' form of SA-MCF, as occurred in this study, seems to be the predominant clinical manifestation of this disease in cattle throughout continental Brazil (Rech *et al.*, 2005; Macêdo *et al.*, 2007; Headley *et al.*, 2012).

In conclusion, pathological alterations consistent with SA-MCF were identified within multiple tissues of two cows and a 4-month-old fetus of one cow. Amplification of OvHV-2 DNA from the brain of the cow and its fetus is likely to indicate involvement of this pathogen in the lesions observed. This study also demonstrated the transplacental transmission of OvHV-2 in SA-MCF of cattle.

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Conflict of Interest Statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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